

## Gram scale laboratory synthesis of TC AC 28, a high affinity BET bromodomain ligand

Article (Published Version)

Khan, Raysa, Marsh, Graham, Felix, Robert, Kemmitt, Paul D, Baud, Matthias G J, Ciulli, Alessio and Spencer, John (2017) Gram scale laboratory synthesis of TC AC 28, a high affinity BET bromodomain ligand. ACS Omega, 2 (8). pp. 4328-4332. ISSN 2470-1343

This version is available from Sussex Research Online: <http://sro.sussex.ac.uk/id/eprint/69509/>

This document is made available in accordance with publisher policies and may differ from the published version or from the version of record. If you wish to cite this item you are advised to consult the publisher's version. Please see the URL above for details on accessing the published version.

### **Copyright and reuse:**

Sussex Research Online is a digital repository of the research output of the University.

Copyright and all moral rights to the version of the paper presented here belong to the individual author(s) and/or other copyright owners. To the extent reasonable and practicable, the material made available in SRO has been checked for eligibility before being made available.

Copies of full text items generally can be reproduced, displayed or performed and given to third parties in any format or medium for personal research or study, educational, or not-for-profit purposes without prior permission or charge, provided that the authors, title and full bibliographic details are credited, a hyperlink and/or URL is given for the original metadata page and the content is not changed in any way.

# Gram-Scale Laboratory Synthesis of TC AC 28, a High-Affinity BET Bromodomain Ligand

Raysa Khan,<sup>†</sup> Graham Marsh,<sup>§</sup> Robert Felix,<sup>§</sup> Paul D. Kemmitt,<sup>‡</sup> Matthias G. J. Baud,<sup>||,⊥</sup> Alessio Ciulli,<sup>||,⊥</sup> and John Spencer<sup>\*,†,⊥</sup>

<sup>†</sup>Department of Chemistry, School of Life Sciences, University of Sussex, Falmer, Brighton BN1 9QJ, U.K.

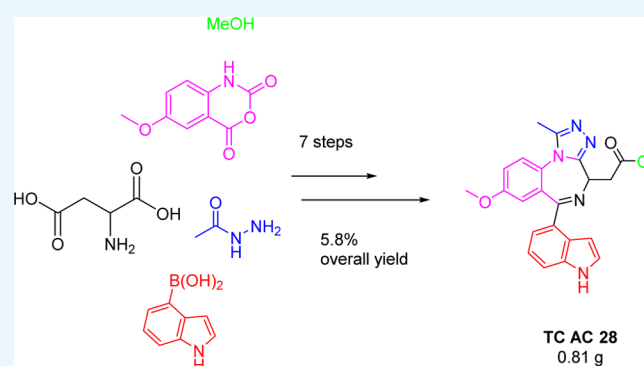
<sup>‡</sup>Oncology, AstraZeneca, 310 Cambridge Science Park, Milton Road, Cambridge CB4 0WG, U.K.

<sup>§</sup>Tocris Bioscience, the Watkins Building, Atlantic Road, Avonmouth, Bristol BS11 9QD, U.K.

<sup>||</sup>Division of Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee, James Black Centre, Dow Street, Dundee DD1 5EH, U.K.

## S Supporting Information

**ABSTRACT:** TC AC 28, 6-(1*H*-Indol-4-yl)-8-methoxy-1-methyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine-4-acetic acid methyl ester, has been synthesized on a near-gram scale in seven steps with notable improvements in the reported poor-yielding last two steps enabling this key chemical probe compound to be available for researchers.



## INTRODUCTION

The 1,4-benzodiazepine scaffold is a well-established “privileged scaffold” in medicinal chemistry,<sup>1–16</sup> and we have an active interest in synthesizing libraries of such compounds.<sup>17–21</sup> Our recently described triazolo-benzodiazepine derivative TC AC 28 is a potent, selective bromo and extraterminal bromodomain inhibitor and a useful epigenetic tool compound, with a crystallographically defined binding mode to the target protein and displaying  $K_d$  values of 40 and 800 nM toward Brd2(2) and Brd2(1), respectively.<sup>22,23</sup> We sought to scale up the original seven-step-protocol toward the racemic product (as in the original manuscript) with the aim of improving the final two problematic and low-yielding steps.<sup>23</sup>

## RESULTS AND DISCUSSION

Our scale-up efforts (step 1, Scheme 1) started with a synthesis of the methyl ester hydrochloride salt 2, which was formed in virtually quantitative yield, followed by a cyclization step (step 2) to afford the isatoic anhydride 4.<sup>24</sup>

Reaction of the latter formed the benzodiazepinedione 5, and we employed an ether trituration, as opposed to our earlier reported chromatographic purification workup. This was followed by treatment with Lawesson’s reagent<sup>25,26</sup> and then mercury-mediated cyclization to afford the triazolo-analogue 7 (steps 3–5). At this stage, no significant differences in yields were noticed from our original report and we did not attempt less toxic routes to 7 given that the yield was acceptable and the

chemistry scalable. However, the next two crucial steps were vital in our aims to obtain approximate gram quantities of product.

Step 6 (Scheme 2) was originally performed by combining 12 batches of ca. 170 mg of precursor 7, producing the key chloroimidate intermediate 8, which was obtained as a white solid in 29% yield (619 mg). Careful reexamination of this step led us to significantly lower the amounts of POCl<sub>3</sub> used, and we were able to avoid the inefficient chromatographic step by carrying out a trituration in Et<sub>2</sub>O (Table 1, entry 3). Indeed, we were delighted to obtain a yield of 76% of 8 in near-gram quantities (0.80 g) in a one-step protocol.

Buoyed by this result, we next examined the final Pd-catalyzed Suzuki–Miyaura coupling reaction to install the indolyl group in 9.<sup>27,28</sup> Maintaining the original Pd(PPh<sub>3</sub>)<sub>4</sub> catalyst, we obtained, by using a 1,2-dimethoxyethane (DME)/water mixture with Na<sub>2</sub>CO<sub>3</sub> as base, 9 in 49% yield (Table 3, entry 2), which was scalable to 0.8 g of product (Table 2).

## CONCLUSIONS

Overall, acceptable, near-gram quantities of the final product 9 have been synthesized, benefitting ultimately from improved steps 6 and 7 of the original synthetic route (Table 3).

Received: June 13, 2017

Accepted: July 25, 2017

Published: August 8, 2017



Scheme 2. Synthesis of TC AC 28 (9)

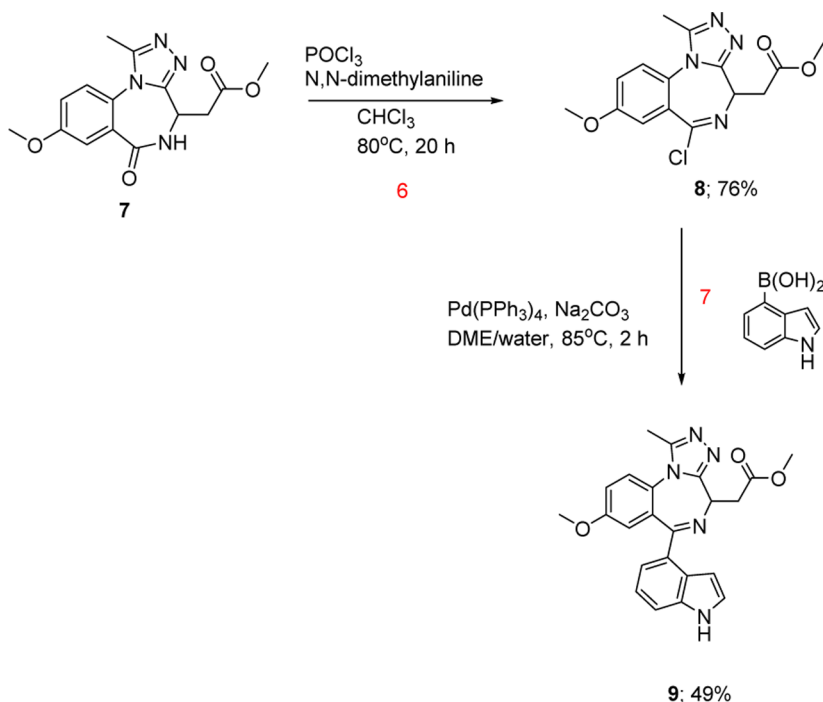


Table 1. Step 6 Optimization

entry	$\text{POCl}_3$ (equiv)	dimethylaniline ( $N,N$ -DMA) (equiv)	workup	purification	isolated yield (8) (%)
1	21	5.5	quench ( $\text{Et}_3\text{N}$ )	acetone/DCM (30–80%) column	20 <sup>a</sup>
2	10	3	quench (water) extraction with $\text{CHCl}_3$	trituration with diethyl ether	50
3	1.5	2	quench (water) extraction with $\text{CHCl}_3$	trituration with diethyl ether	76

<sup>a</sup>Material decomposes on silica.

Table 2. Suzuki Coupling Optimization

entry	catalyst	solvent	base	conditions	isolated yield (9) (%)
1	$\text{Pd(PPh}_3)_4$	dimethylformamide	$\text{Et}_3\text{N}$	$100^\circ\text{C}$ , 24 h	27
2	$\text{Pd(PPh}_3)_4$	DME/water	$\text{Na}_2\text{CO}_3$	$85^\circ\text{C}$ , 2 h	49

Table 3. Comparison of Scale-Up vs Original Published Route

step	S.M. (g) <sup>a</sup>	prod. (g)	yield (%)	S.M. (g) <sup>b</sup>	prod. (g)	yield (%)
1	50.07	74.00	>99			
2	50.02	57.03	89			>99
3	45.00	27.30	43 <sup>c</sup>	3.70	1.77	36
4	15.01	8.30	53	1.86	1.12	57
5	8.00	6.57	77 <sup>d</sup>	2.20	2.15	91
6	0.99	0.80	76 <sup>e</sup>	2.04 (0.17 × 12)	0.619	29
7	1.33	0.81	49			27–31

<sup>a</sup>Scale-up (this work); S.M. = starting material, prod. = product.<sup>b</sup>Original papers. <sup>c</sup>Trituration in ether as opposed to chromatography.<sup>d</sup>Reaction mixture quenched with  $\text{NaHCO}_3$ , extracted with ethyl acetate as opposed to no workup. <sup>e</sup> $\text{POCl}_3$  (1.5 equiv), DMA (2 equiv) quenched with water, extraction with  $\text{CHCl}_3$ , and trituration with diethyl ether as opposed to  $\text{POCl}_3$  (21 equiv), DMA (5.5 equiv), quenched with  $\text{Et}_3\text{N}$  and purified by chromatography.

by filtration and dried at  $50^\circ\text{C}$  under vacuum, affording the product as a brown solid (17.00 g, 89%). LCMS purity (UV):

99%,  $t_R$  3.24 min. The NMR data were consistent with those reported.<sup>23</sup>

**Methyl-2-(7-methoxy-2,5-dioxo-2,3,4,5-tetrahydro-1H-benzo[e][1,4]diazepin-3-yl)acetate (5).** 5-Methoxyisatoic anhydride 4 (45.00 g, 232.97 mmol) and DL-aspartic acid dimethyl ester hydrochloride (46.04 g, 232.99 mmol, 1 equiv) were suspended in pyridine (600 mL), and the reaction mixture was stirred at reflux for 18 h. After cooling to ambient temperature, the reaction mixture was concentrated under reduced pressure. The residue was partitioned between ethyl acetate (500 mL) and 2 M HCl (500 mL). The organic layer was separated, and the aqueous layer was further extracted with ethyl acetate (2 × 350 mL). Some solid material at the phase boundary was collected by filtration, giving an initial crop of product. The combined organic phase of the filtrate was dried ( $\text{MgSO}_4$ ) and concentrated under reduced pressure. Trituration with diethyl ether afforded the product as a white solid (27.30 g, 43%). LCMS purity (UV): 96%,  $t_R$  3.12 min. The NMR data were consistent with those reported.<sup>23</sup>

**(+/-)-Methyl-2-(7-methoxy-5-oxo-2-thioxo-2,3,4,5-tetrahydro-1H-benzo[e][1,4]diazepin-3-yl)acetate (6).** To a suspension of the previous compound 5 (15.01 g, 53.91

mmol) in pyridine (265 mL), Lawesson's reagent (19.62 g, 48.52 mmol, 0.9 equiv) was added, and the reaction mixture was stirred at reflux for 6 h. The reaction mixture was cooled to ambient temperature and concentrated under reduced pressure. The residue was suspended in  $\text{CH}_2\text{Cl}_2$  ( $3 \times 300$  mL) and re-concentrated under reduced pressure. Trituration with  $\text{CH}_2\text{Cl}_2$  afforded the product as a pale yellow solid (8.30 g, 53%). LCMS purity (UV): 92%,  $t_R$  3.51 min. The NMR data were consistent with those reported.<sup>23</sup>

**(+/-)-Methyl-2-(8-methoxy-1-methyl-6-oxo-5,6-dihydro-4H-benzo[f][1,2,4]triazolo[4,3-a][1,4]diazepin-4-yl)-acetate (7).** To a stirred suspension of compound 6 (8.00 g, 27.18 mmol) and acethydrazide (6.04 g, 81.53 mmol, 3 equiv) in THF (120 mL), acetic acid (80 mL) was added. The reaction mixture was cooled to 0 °C, and mercury (II) acetate (12.91 g, 40.77 mmol, 1.5 equiv) was added to the reaction mixture portionwise at such a rate that the temperature was maintained below 5 °C. Upon completion of the addition, the reaction mixture was stirred at 0 °C for 2 h and then allowed to warm to ambient temperature and stirred for 48 h. The reaction mixture was concentrated under reduced pressure, and the residue was partitioned between  $\text{NaHCO}_3$  (sat. aq., 450 mL) and ethyl acetate (300 mL). The aqueous component was separated and extracted with ethyl acetate ( $2 \times 300$  mL). The combined organic layer was dried ( $\text{MgSO}_4$ ) and concentrated under reduced pressure. The product was collected as a white solid (6.57 g, 77%) after flash column chromatography (95:5  $\text{CH}_2\text{Cl}_2/\text{MeOH}$ ). LCMS purity (UV): 95%,  $t_R$  3.15 min. The NMR data were consistent with those reported.<sup>23</sup>

**(+/-)-Methyl-2-(6-chloro-8-methoxy-1-methyl-4H-benzo[f][1,2,4]triazolo[4,3-a][1,4]diazepin-4-yl)acetate (8).** To a solution of compound 7 (0.99 g, 3.13 mmol) in  $\text{CHCl}_3$  (20 mL), *N,N*-dimethylaniline (0.79 g, 6.26 mmol) and  $\text{POCl}_3$  (0.72 g, 4.70 mmol) were added under inert atmosphere, and the reaction was heated at 80 °C for 18 h. After cooling to room temperature, the reaction was slowly poured into lukewarm water (80 mL) with stirring. After stirring for 15 min, it was diluted with  $\text{CHCl}_3$  (50 mL) and the layers were separated. The aqueous layer was extracted with further  $\text{CHCl}_3$  (50 mL). The combined organic layer was dried ( $\text{MgSO}_4$ ) and concentrated under reduced pressure. The residue was triturated with diethyl ether to afford an off-white solid (0.80 g, 76%). The product was used without further purification. LCMS purity (UV): 97%,  $t_R$  3.94 min. The NMR data were consistent with those reported.<sup>23</sup>

**(+/-)-Methyl-2-(6-chloro-8-methoxy-1-methyl-4H-benzo[f][1,2,4]triazolo[4,3-a][1,4]diazepin-4-yl)acetate (9).** To a stirred suspension of compound 8 (1.33 g, 3.97 mmol) in DME (14 mL), a solution of  $\text{Na}_2\text{CO}_3$  (0.76 g, 7.17 mmol) in water (6 mL) was added, followed by the addition of indole-4-boronic acid (0.77 g, 4.76 mmol) and  $\text{Pd}(\text{PPh}_3)_4$  (0.31 g, 0.27 mmol), and the reaction was heated at 85 °C for 2.5 h. After cooling to ambient temperature, it was filtered over celite, and the filtrate was partitioned between  $\text{EtOAc}/\text{water}$ . The layers were separated, and the organic layer was further washed with water and brine. The organic layer was dried ( $\text{MgSO}_4$ ) and concentrated under reduced pressure. The product was collected as a white solid (0.81 g, 49%) after flash column chromatography ( $r_f = 0.35$ ; 95:5  $\text{CH}_2\text{Cl}_2/\text{MeOH}$ ).  $^1\text{H}$  NMR (400 MHz)  $\text{CDCl}_3$ :  $\delta = 8.40$  (s, 1H), 7.52 (d,  $J = 8.0$ , 1H), 7.42 (d,  $J = 9.0$ , 1H), 7.24 (t,  $J = 3.0$ , 1H), 7.20 (dd,  $J = 3.0$ ,  $J = 9.0$ , 1H), 7.15 (t,  $J = 7.5$ , 1H), 7.08 (d,  $J = 7.5$ , 1H), 6.92 (d,  $J = 3.0$ , 1H), 6.58 (s, 1H), 4.78 (dd,  $J = 5.5$ ,  $J = 9.0$ , 1H), 3.81 (s, 3H),

3.72–3.78 (m, 4H), 3.63 (dd,  $J = 5.5$ ,  $J = 16.5$ , 1H), 2.64 (s, 3H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta = 172.5$ , 168.1, 157.9, 156.4, 150.5, 136.5, 131.9, 130.8, 126.9, 126.4, 125.5, 124.3, 123.4, 121.2, 117.7, 116.5, 113.6, 103.1, 55.8, 53.4, 51.9, 36.9, 12.2. LCMS purity (UV): 99%,  $t_R$  4.12 min. Elemental analysis: calcd for  $\text{C}_{23}\text{H}_{21}\text{N}_5\text{O}_3 \cdot 3/4\text{H}_2\text{O}$  (%): C, 64.40, H, 5.29, N, 16.33, found: C, 64.73, H, 5.12, N, 16.07. MS  $m/z$  (ES+) calculated for  $\text{C}_{23}\text{H}_{21}\text{N}_5\text{O}_3$   $[\text{+H}]^+$ : 416.3 found: 416.3;  $m/z$  (ES-) calcd for  $\text{C}_{23}\text{H}_{21}\text{N}_5\text{O}_3$   $[-\text{H}]^+$ : 414.3 found: 414.3.

## ■ ASSOCIATED CONTENT

### § Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b00780.

Scanned NMR spectra and HPLC purity for all compounds (PDF)

## ■ AUTHOR INFORMATION

### Corresponding Author

\*E-mail: j.spencer@sussex.ac.uk.

### ORCID

Matthias G. J. Baud: 0000-0003-3714-4350

Alessio Ciulli: 0000-0002-8654-1670

John Spencer: 0000-0001-5231-8836

### Present Address

<sup>†</sup>Chemistry Department, Faculty of Natural and Environmental Sciences, University of Southampton, Southampton SO17 1BJ, U.K. (M.G.J.B.).

### Author Contributions

All authors have given approval to the final version of the manuscript.

### Funding

A.C. and M.G.J.B. were funded by BBSRC grants BB/G023123/2 and BB/J001201/2. R.K. is funded by an EPSRC/AZ-funded iCASE PhD studentship (EP/M507568/1).

### Notes

The authors declare the following competing financial interest(s): the title product, **TC AC 28**, is sold under license from the University of Dundee and is available at Tocris on: <https://www.tocris.com/dispprod.php?ItemId=519094#.WSHyEU3rviU>.

## ■ ACKNOWLEDGMENTS

Tocris is acknowledged for providing addition support for this project.

## ■ ABBREVIATIONS

TLC, thin-layer chromatography; *N,N*-DMA, dimethylaniline

## ■ REFERENCES

- (1) Ellman, J. A. Design, Synthesis, and evaluation of small-molecule libraries. *Acc. Chem. Res.* **1996**, *29*, 132–143.
- (2) Chung, C. W.; Coste, H.; White, J. H.; Mirguet, O.; Wilde, J.; Gosmini, R. L.; Delves, C.; Magny, S. M.; Woodward, R.; Hughes, S. A.; Boursier, E. V.; Flynn, H.; Bouillot, A. M.; Bamborough, P.; Brusq, J. M.; Gellibert, F. J.; Jones, E. J.; Riou, A. M.; Homes, P.; Martin, S. L.; Uings, I. J.; Toum, J.; Clement, C. A.; Boullay, A. B.; Grimley, R. L.; Blandel, F. M.; Prinjha, R. K.; Lee, K.; Kirilovsky, J.; Nicodeme, E. Discovery and characterization of small molecule inhibitors of the BET family bromodomains. *J. Med. Chem.* **2011**, *54*, 3827–3838.



- (3) Evans, B. E.; Rittle, K.; Bock, M.; DiPardo, R.; Freidinger, R.; Whitter, W.; Lundell, G.; Veber, D.; Anderson, P.; Chang, R.; Lotti, V.; Cerino, D.; Chen, T.; Kling, P.; Kunkel, K.; Springer, J.; Hirshfield, J. Methods for drug discovery: development of potent, selective, orally effective cholecystokinin antagonists. *J. Med. Chem.* **1988**, *31*, 2235–2246.
- (4) Smith, S. G.; Zhou, M. M. The Bromodomain: a new target in emerging epigenetic medicine. *ACS Chem. Biol.* **2016**, *11*, 598–608.
- (5) Nicodeme, E.; Jeffrey, K. L.; Schaefer, U.; Beinke, S.; Dewell, S.; Chung, C. W.; Chandwani, R.; Marazzi, I.; Wilson, P.; Coste, H.; White, J.; Kirilovsky, J.; Rice, C. M.; Lora, J. M.; Prinjha, R. K.; Lee, K.; Tarakhovskiy, A. Suppression of inflammation by a synthetic histone mimic. *Nature* **2010**, *468*, 1119–1123.
- (6) Filippakopoulos, P.; Knapp, S. Targeting bromodomains: epigenetic readers of lysine acetylation. *Nat. Rev. Drug Discovery* **2014**, *13*, 337–356.
- (7) Filippakopoulos, P.; Picaud, S.; Fedorov, O.; Keller, M.; Wrobel, M.; Morgenstern, O.; Bracher, F.; Knapp, S. Benzodiazepines and benzotriazepines as protein interaction inhibitors targeting bromodomains of the BET family. *Bioorg. Med. Chem.* **2012**, *20*, 1878–1886.
- (8) Abdelkafi, H.; Cintrat, J. C. Regioselective Halogenation of 1,4-Benzodiazepinones via CH Activation. *Sci. Rep.* **2015**, *5*, No. 12131.
- (9) Field, G. F.; Zally, W. J.; Sternbach, L. H. Quinazolines and 1,4-benzodiazepines. XXXIII. Three tautomeric forms of the benzodiazepine ring system. *J. Am. Chem. Soc.* **1967**, *89*, 332–338.
- (10) Dziadulewicz, E. K.; Brown, M. C.; Dunstan, A. R.; Lee, W.; Said, N. B.; Garratt, P. J. The design of non-peptide human Bradykinin B2 receptor antagonists employing the benzodiazepine peptidomimetic scaffold. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 463–468.
- (11) Guandalini, L.; Cellai, C.; Laurenzana, A.; Scapecchi, S.; Paoletti, F.; Romanelli, M. N. Design, synthesis and preliminary biological evaluation of new hydroxamate histone deacetylase inhibitors as potential antileukemic agents. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5071–5074.
- (12) Ghelani, S. M.; Naliapara, Y. T. Design, synthesis, and characterization of 1, 3-disubstituted-1,4-benzodiazepine derivatives. *J. Heterocycl. Chem.* **2016**, *53*, 1795–1800.
- (13) El Bouakher, A.; Prié, G.; Aadil, M.; Akssira, M.; Viaud-Massuard, M.-C. Synthesis of novel 2,4-disubstituted 3H-pyrido[3,2-e][1,4]diazepin-5-ones and 2,4-disubstituted 3H-pyrido[2,3-e][1,4]diazepin-5-ones derivatives via regioselective thionation and nucleophilic substitutions reactions. *Tetrahedron* **2013**, *69*, 5086–5091.
- (14) Kaur, N. Recent trends in the chemistry of privileged scaffold: 1,4-benzodiazepine. *Int. J. Pharma Bio Sci.* **2013**, *4*, 485–513.
- (15) Hu, X.; Dong, Y.; Liu, G. Copper-catalyzed ligand-free amidation of aryl iodides and amino acid amides to synthesize C3-(Z)-1H-benzo[e][1,4]diazepin-2(3H)-ones. *Mol. Diversity* **2015**, *19*, 695–701.
- (16) Taylor, A. M.; Vaswani, R. G.; Gehling, V. S.; Hewitt, M. C.; Leblanc, Y.; Audia, J. E.; Bellon, S.; Cummings, R. T.; Cote, A.; Harmange, J. C.; Jayaram, H.; Joshi, S.; Lora, J. M.; Mertz, J. A.; Neiss, A.; Pardo, E.; Nasveschuk, C. G.; Poy, F.; Sandy, P.; Setser, J. W.; Sims, R. J., 3rd; Tang, Y.; Albrecht, B. K. Discovery of benzotriazolo-[4,3-d][1,4]diazepines as orally active inhibitors of BET bromodomains. *ACS Med. Chem. Lett.* **2016**, *7*, 145–150.
- (17) Spencer, J.; Chowdhry, B. Z.; Mallet, A. I.; Rathnam, R. P.; Adatia, T.; Bashall, A.; Rominger, F. C–H activations on a 1H-1,4-benzodiazepin-2(3H)-one template. *Tetrahedron* **2008**, *64*, 6082–6089.
- (18) Spencer, J.; Rathnam, R.; Chowdhry, B. 1,4-Benzodiazepin-2-ones in medicinal chemistry. *Future Med. Chem.* **2010**, *2*, 1441–1449.
- (19) Spencer, J.; Rathnam, R. P.; Harvey, A. L.; Clements, C. J.; Clark, R. L.; Barrett, M. P.; Wong, P. E.; Male, L.; Coles, S. J.; Mackay, S. P. Synthesis and biological evaluation of 1,4-benzodiazepin-2-ones with antitrypanosomal activity. *Bioorg. Med. Chem.* **2011**, *19*, 1802–1815.
- (20) Clark, R. L.; Clements, C. J.; Barrett, M. P.; Mackay, S. P.; Rathnam, R. P.; Owusu-Dapaah, G.; Spencer, J.; Huggan, J. K. Identification and development of the 1,4-benzodiazepin-2-one and quinazoline-2,4-dione scaffolds as submicromolar inhibitors of HAT. *Bioorg. Med. Chem.* **2012**, *20*, 6019–6033.
- (21) Khan, R.; Felix, R.; Kemmitt, P. D.; Coles, S. J.; Day, I. J.; Tizzard, G. J.; Spencer, J. Late Stage c-h activation of a privileged scaffold. synthesis of a library of benzodiazepines. *Adv. Synth. Catal.* **2016**, *358*, 98–109.
- (22) Baud, M. G. J.; Lin-Shiao, E.; Cardote, T.; Tallant, C.; Pschibul, A.; Chan, K. H.; Zengerle, M.; Garcia, J. R.; Kwan, T. T.; Ferguson, F. M.; Ciulli, A. Chemical biology. A bump-and-hole approach to engineer controlled selectivity of BET bromodomain chemical probes. *Science* **2014**, *346*, 638–641.
- (23) Baud, M. G.; Lin-Shiao, E.; Zengerle, M.; Tallant, C.; Ciulli, A. New synthetic routes to triazolo-benzodiazepine analogues: expanding the scope of the bump-and-hole approach for selective bromo and extra-terminal (BET) bromodomain inhibition. *J. Med. Chem.* **2016**, *59*, 1492–1500.
- (24) Carter, M. C.; Alber, D. G.; Baxter, R. C.; Bithell, S. K.; Budworth, J.; Chubb, A.; Cockerill, G. S.; Dowdell, V. C. L.; Henderson, E. A.; Keegan, S. J.; Kelsey, R. D.; Lockyer, M. J.; Stables, J. N.; Wilson, L. J.; Powell, K. L. 1,4-Benzodiazepines as inhibitors of respiratory syncytial virus. *J. Med. Chem.* **2006**, *49*, 2311–2319.
- (25) Hitotsuyanagi, Y.; Motegi, S.; Fukaya, H.; Takeya, K. A cis amide bond surrogate incorporating 1,2,4-triazole. *J. Org. Chem.* **2002**, *67*, 3266–3271.
- (26) Syeda, S. S.; Jakkaraj, S.; Georg, G. I. Scalable syntheses of the BET bromodomain inhibitor JQ1. *Tetrahedron Lett.* **2015**, *56*, 3354–3457.
- (27) Nadin, A.; Sánchez López, J. M.; Owens, A. P.; Howells, D. M.; Talbot, A. C.; Harrison, T. New synthesis of 1,3-dihydro-1,4-benzodiazepin-2(2H)-ones and 3-amino-1,3-dihydro-1,4-benzodiazepin-2(2H)-ones: Pd-catalyzed cross-coupling of imidoyl chlorides with organoboronic acids. *J. Org. Chem.* **2003**, *68*, 2844–2852.
- (28) Glick, G. D. Benzodiazepinone Compounds Useful in the Treatment of Skin Conditions. U.S. Patent US 20090118244 A1, Nov 6, 2008.
- (29) Fowler, L. S.; Ellis, D.; Sutherland, A. Synthesis of fluorescent enone derived alpha-amino acids. *Org. Biomol. Chem.* **2009**, *7*, 4309–4316.